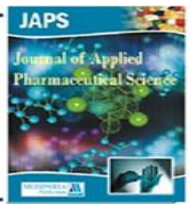
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 03-08-2012
Revised on: 19-08-2012
Accepted on: 24-08-2012
DOI: 10.7324/JAPS.2012.2825

Hepatoprotective Activity of Methanolic Extract of *Rhyncosia Beddomei* Baker Leaves Against Carbon Tetrachloride Induced Hepatotoxicity

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ABSTRACT

Rhyncosia beddomei Baker commonly known as Adavi-kandi, Vendiaku in Telugu belongs to the family Fabaceae.In the present study, the methanolic extract of *Rhyncosia beddomei* leaves *was* evaluated for its hepatoprotective effect against CCl_4 induced hepatic injury in rats. Alteration in the levels of biochemical markers of hepatic damage like SGOT, SGPT, ALP, triglycerides, bilirubin, total proteins and liver weight were tested in both treated and untreated groups. CCl_4 (1ml/kg) enhanced the SGPT, SGOT, ALP, triglycerides, liver weight and reduced total proteins significantly. Treatment with methanolic extract of *Rhyncosia beddomei* leaves (200mg/kg and 400mg/kg) has brought back the altered levels of altered levels of biochemical markers significantly to the near normal levels in the dose dependant manner. Histopathological studies supported the hepatoprotective activity of *Rhyncosia beddomei* Baker.

Keywords: *Rhyncosia beddomei*, Hepatoprotective activity, Biochemical markers, SGOT, SGPT, ALP

INTRODUCTION

Liver plays an major role in detoxification and excretion of many endogenous and exogenous compounds and injury to liver or impairment of its functions may lead to many implications on one's health(Handa and Kapoor, 2002).Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition to the above serum levels of many biochemical markers like SGPT, SGOT, ALP, triglycerides, cholesterol, bilirubin are elevated and total proteins depleted (Ramachandra *et al.*, 2007). Hepatic disorders have been recognized worldwide as an important cause of morbidity and mortality in man and animals all over the globe. Hepato toxicity of drugs appears to be the most common contributing factor (Sangameswaran *et al.*, 2008). Herbal medicines are known to play an important role in the treatment of various elements including liver disorders and many traditional practioners have claimed that numerous medicinal plants can be extensively used for the alleviation of different types of liver disorders (Dash *et al.*, 2007). Inspite of phenomenal growth of modern medicine there are no synthetic drugs are available for the treatment of hepatic disorders. However there are several herbs/herbal formulation claimed have posses beneficial activity in treating hepatic disorders.

Rhyncosia beddomei Baker commonly known as Adavi-kandi, Vendiaku,Vendaku in Telugu belongs to the family fabaceae, mainly found in Eastern Ghats of Andhra Pradesh, India. The leaves are reported to contain flavanoids, alkaloids, glycosides, lignans, tri terpenoids and reported to be useful as abortifacient, antibacterial, anti diabetic and hepatoprotective. Leaves are also used for wounds, cuts, boils and rheumatic pains by adivasi tribes (Chetty *et al.,* 2008; Rastogi and Mehrotra, 1970). The traditional uses and phytoconstituents of *Rhyncosia beddomei* Baker prompt us to take up this study.

MATERIALS AND METHODS

Plant material

The plant material was collected from vicinity of Tirumala hills, Chittor district of Andhra Pradesh, identified and authenticated by Dr. Madhava chetty, Asst.Professor, Botany Dept, Sri Venkateswara University, Tirupati. Herbarium is deposited in herbal drug museum of M.S.Ramaiah College of Pharmacy, Bangalore.

Preparation of plant extracts

The roots were collected, washed and dried at room temperature. After complete drying, it was powdered in a multi mill grinder and passed through a 60 mesh sieve. Dried powdered drug was subjected to successive solvent extraction (petroleum ether, benzene, chloroform, methanol and water) .

Phytochemical Screening

Extracts obtained on successive solvent extraction were subjected to phytochemical screening for the detection of various phytosconstituents (Kokate, 1999).

ANIMAL STUDIES

Experimental animals

The pharmacological studies were carried out on Albino Wister rats of either sex weighing 150-225 g. The animals were housed in the animal house of MSRCP and maintained in controlled temperature (27 ± 2^0C) and light cycle (12 hr light and 12 hr dark). They were fed with rat feed (rat pellets from VRK Nutritional solutions, Sangli, Maharashtra, India) and water ad libitum. The study protocol was approved by the institutional Animal Ethical Committee of MSRCP (IAEC certificate No: MSRCP/P- 2010, Dated 3/12/2010).

Acute toxicity studies(OECD 423)

An acute toxicity study was performed on methanol extract following OECD guidelines (423). The dosage for the pharmacological studies was selected as 1/10th of the highest dose (2000mg/kg) administered.

Experimental design (Krishna *et al.,* 2010)

Rats were divided into 5 groups 6 animals each as follows: Group I served as vehicle control and received oral administration of distilled water containing 2% gum acacia. Group II served as positive control and received oral administration of vehicle plus CCl_4 (1ml/kg body weight). Group III served as standard group and received silymarin (100mg/kg body weight p.o.) once daily for 7 days. Group IV and V were orally administered with methanol extract of drug at the dose of 200mg and 400 mg /kg respectively once daily for 7 days. On the 7^{th} day, all groups except group I, were given a single dose of CCl4 (1ml/kg body weight p.o.) in 1:1 liquid paraffin after 6 hrs of last dose administration. On the 8^{th} day, 18 h after the dose of CCl_4, all the animals were anaesthetized under light ether anastasia and the blood was collected from retro orbital sinus using a heperinized capillary tube.

Isolation of liver

Liver was carefully excised and washed in ice cold normal saline solution and pressed between filter paper pads and weighed. A portion of liver (one animal of each group) was preserved in 10% neutral formalin for histopathology studies.

BIOCHEMICAL ESTIMATION

Blood was allowed to clot and centrifuged at 12000 rpm for 10 min to separate the serum. The serum thus obtained was used for the estimation of Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxalloacetate transaminase (SGOT) (Bergmeyer *et al*., 1977), alkaline phosphatase (ALP) (Bessey *et al.,* 1946), tri glycerides (Bucolo, 1973), total proteins (Henry *et al.,* 1974) and bilirubin (Pearlman, and Lee, 1974). All these estimations were performed following International Federation of Clinical chemistry and Laboratory medicine (IFCC) standard procedures. Isolated serum was used for estimating SGPT, SGOT, ALP, total proteins, triglycerides and bilirubin. All the determinations were carried out using standard kits (Agappe diagnostics, Beacon Diagnostics, Apparechi Diagnostics) by using Semi-automatic B4B Diagnostic Division Chemistry Analyzer CA-2005 Ranbaxy diagnostic division

HISTOPATHOLOGY STUDIES (Nanji *et al.,* 2001)

Paraffin sections were prepared from formalin fixed liver samples and stained with haematoxylin and eosin. Histological samples were categorized based on the extent of hepatic injury (necrosis, inflammation, fibrosis, vascular characteristics and overall injury)

STATISTICAL ANALYSIS

All values are expressed as Mean± SEM and tested with One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of different extracts was carried out to obtain the information about presence of

various phytoconstituents and methanolic extract found to contain carbohydrates, flavonoids, phenolic compounds and tannins. Alkaloids, flavonoids and saponins known to posses hepatoprotective activity (Vijayan *et al.,* 2001) and hence the methanolic extract was selected in this study.

Acute toxicity studies of methanolic extract at the dose of 300mg/kg and 2000mg/kg showed no toxic symptoms or death in any of the animals upto one week and till the end of the study. Thus the drug was considered to be safe.

The marker enzyme levels in different group of animals are shown in the Table1. The liver weight and serum levels of SGPT, SGOT, ALP, triglycerides and bilirubin were increased significantly while that of total proteins decreased in positive control group. The treatment with the extract altered serum parameters significantly to the normal values.

The serum levels of SGPT and SGOT were significantly ($P<0.001$ for 200mg/kg and $P<0.001$ for 400 mg/kg) reduced in the extract treated group. The serum levels of ALP were also significantly ($P<0.01$ for 200mg/kg and $P<0.001$ 400 mg/kg) reduced in the extract treated group. Tri glyceride levels significantly reduced for 400 mg/kg ($P<0.01$), but non significant for 200mg/kg dose. Total protein levels were significantly increased ($P<0.001$ for 200mg/kg and 400 mg/kg) and bilirubin levels were significantly reduced ($P<0.001$ for 200mg/kg and 400 mg/kg) in the extract treated group. The extract of 200mg/kg does not show any significant effect on liver weight, but 400mg/kg reduced significantly ($P<0.01$).

Liver photomicrographs of different groups shown in figure 1. Normal liver control showed normal hepatic architecture with portal tracts, central veins, hepatocytes and sinusoids. Positive control group showed loss of normal liver architecture with Degenerative hepatocytes, fibrosis, Sinusoidal spaces with inflammatory cells, ballooning of cells and centri lobular necrosis. Liver photomicrograph of drug extract (200mg/kg) showed mild fibrosis**,** light hepatocyte regeneration and ballooning of hepatocytes, where as drug extract (400mg/kg) showed minimal fibrosis, regeneration of hepatocytes and ballooning of hepatocytes. Treatment with standard Silymarin showed almost normal liver architecture. The liver is major organ involved in various metabolic functions and detoxification of hazardous substances. Liver diseases remain as one of the major health problems and no satisfactory allopathic drug for the treatment is available so for. Herbal drugs play a major role in the management of various liver disorders in addition to other healing processes of the liver (Subramonioum *et al.,* 1998). Earlier studies have demonstrated the use of carbon tetra chloride to induce hepatotoxicity in experimental animals. The toxin CCl_4 is biotransformed by cytochrome P-450 to produce trichloro-methyl radical, which leads to peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. Trichloro methyl free radicals elicit lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. All these event result in loss of integrity of the cell membranes and hepatic tissue damage (Vadivu *et al.,* 2008).

Amino transferases SGPT and SGOT catalyze the interconversion of amino acids and α-keto acids by the transfer of an amino group. These enzymes are very sensitive and are reliable indicies for hepatoprotective or curative effects of various compounds (Heyes *et al.,* 1986). Alkalline phosphatase (ALP) is produced by bone, liver, intestine, placenta and is also excreted in the bile. In the absence of bone disease and pregnancy, there is an elevated serum ALP levels due to increased production of ALP by hepatic parenchymal or duct cells (Kind and King, 1954). Bilirubin, a metabolic product of the breakdown of heme rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum or in hemolysis (Harsh Mohan, 2001). Elevated levels of SGPT, SGOT, ALP and bilirubin were observed in positive control group and were reduced significantly in all drug treated groups. Liver cells synthesize various proteins like albumin, fibrinogen, haptoglobin, transferrin and antitrypsin. The blood levels of these proteins are decreased in extensive liver damage. Serum proteins levels were found to decrease in positive control group which was reversed in extract treated group. Serum enzyme levels are not a direct measure of hepatic injury, but elevated levels are indicative of cellular leakage and loss of integrity of cell membrane. Thus lowering of enzyme content in serum is a definite indication of hepatoprotection of the drug. The results were further supported by histopathological studies substantiating the use of leaves of *Rhyncosia beddomei* Baker as a potential hepatoprotective drug.

Table 1: Effect of *Rhyncosia beddomei* Baker leaves on serum parameters for CCl_4 induced hepatotoxicity.

Groups	Liver wt (g/100g bw)	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total proteins (g/dl)	Total bilirubin (mg/dl)	Triglyceride (mg/dl)
Normal control	3.631±0.153	51.61±4.24	99.73±7.09	151.71±6.0	9.137±0.786	0.483±0.127	83.84±5.88
Positive control	4.369±0.126	282.75±8.24	325.9±26.4	346.78±16	6.157±0.734	2.5±0.2706	193.51±25.4
Standard (Silymarin)	3.578±0.112*	123.18±8.58 ***	126.05±20.9 ***	144.8±11.9 ***	8.847±0.352 ***	0.571±0.12 ***	94.108±2.86 ***
Rb extract (200mg)	4.105±0.0139 ns	187.35±9.85 ***	196.27±8.8 ***	280.9±10.4 **	7.848±0.414 ***	1.188±0.087 ***	167.25±21.7 ns
Rb extract (400mg)	3.78±0.125 **	123.2±19.08 ***	149.45±21.2 ***	244.4±7.46 ***	8.603±0.471 ***	0.838±0.008 ***	102.87±6.5 ***

Rb= *Rhyncosia beddomei* Baker

Values are expressed as Mean ± SEM; Data is compared against positive control group. One way analysis of variance (ANOVA) Tukey-Kramer multiple comparisons test. *** P< 0.001, ** P< 0.01, * P< 0.05.

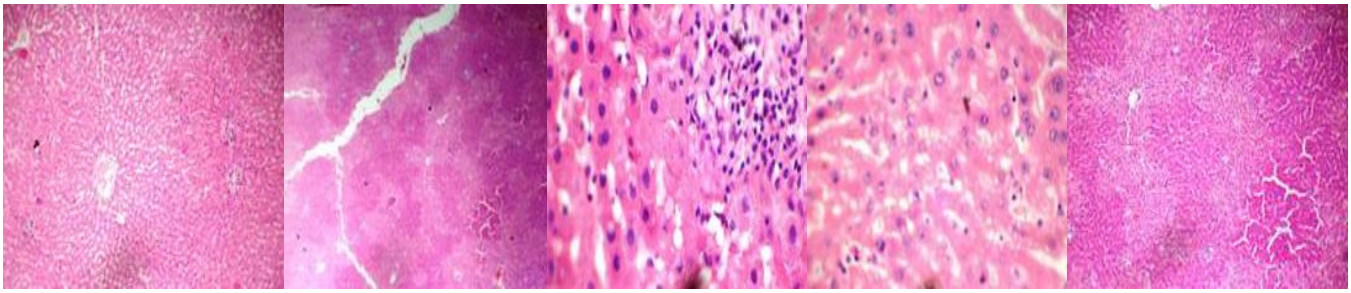

Fig 1. Histopathology of liver samples.

CONCLUSION

The results obtained in the present study indicated that the methanolic extract of leaves of *Rhyncosia beddomei* Baker posses significant hepatoprotective activity. The hepatoprotective action of leaves of *Rhyncosia beddomei* Baker may be due to the presence of phytoconstituents like flavonoids and phenolic compounds.

ACKNOWLEDGEMENT

The authors are thankful to Gokula education foundation, Bangalore for providing facilities to carry out the research work

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